used by Lee et al., in combination with large-scale profiling of individual patients (Chen et al., 2012), will drive the discovery of powerful effective drug combinations and new therapeutic strategies. Once we learn how cells become rewired and reach new network states, it will be much more feasible to force tumor cells out of these pathological states in order to kill or "normalize" them through drug-induced dynamic rewiring of signaling networks. It remains to be seen whether these approaches will be effective in clinical trials. But for now, the future looks very bright for network medicine.

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RNP Export by Nuclear Envelope Budding

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Nuclear export of mRNAs is thought to occur exclusively through nuclear pore complexes. In this issue of *Cell*, Speese et al. identify an alternate pathway for mRNA export in muscle cells where ribonucleoprotein complexes involved in forming neuromuscular junctions transit the nuclear envelope by fusing with and budding through the nuclear membrane.

The canonical model of macromolecular trafficking between the nucleus and the cytoplasm stipulates that all communication between these compartments occurs exclusively via the nuclear pore complexes (NPCs), which perforate the double membrane of the nuclear envelope (NE). Import and export through the NPCs relies on specific signal sequences and associated transport factors. Regulated transport through the NPCs maintains proper gene expression by ensuring that only correctly formed mRNAs access the translation machinery in the cytoplasm. In this issue of *Cell*, Speese et al. (2012) add a new wrinkle to the process and show that large RNP granules formed in *Drosophila* muscle cells during synaptogenesis are exported by budding through the NE instead of passing through the NPCs. (Figure 1).

Newly transcribed mRNAs assemble into large ribonucleoprotein complexes prior to export from the nucleus. These particles vary in size and some exceed the NPC diameter (Grünwald et al., 2011). One of the largest known RNPs, the Balbiani ring particle, overcomes this difficulty by undergoing extensive remodeling to conform to the NPC diameter (Daneholt, 2001). In addition, certain viruses export large particles by using a mechanism that completely bypasses the NPCs. Herpes viruses assemble large capsids in the nucleus that bind to the inner nuclear membrane, dissolve the lamina, a protein network that supports the nucleus, and bud into the perinuclear space between the inner and outer nuclear membranes (INM and ONM). From there the capsid buds fuse with the ONM to exit to the cytoplasm (Johnson and Baines, 2011). Now, Speese and colleagues suggest that the virus may have co-opted a mechanism already used for RNP transport.

Signaling at neuromuscular junctions (NMJs) during *Drosophila* development involves the internalization and cleavage of the wnt receptor DFrizzled2. Processing of DFrizzled2 to DFz2C is required for proper synapse formation, but how

this C-terminal deletion product functions is unclear. DFz2C localizes to large intranuclear foci and the authors identified components of these foci and proteins regulating their formation. They found that these foci are in fact RNP granules containing mRNAs important for NMJ formation and that localization of Par6 mRNA to the synapse was abrogated when foci formation was blocked.

Examination of DFz2C foci showed that they always localized to the nuclear periphery and were surrounded by lamin C (lamC), which is one of two lamins comprising the nuclear lamina in *Drosophila*. LamC was required for RNP granule formation and, consequently, proper synapse formation. Interestingly, DFz2C was required for the relocalization of lamC to foci, suggesting that granule assembly regulates the lamC network. In support of this, aPKC, an atypical PKC kinase present in the nucleus, was found to phosphorylate lamC and be required for the re-localization of lamC to foci.

PKC phosphorylation of lamins contributes to lamina disassembly in both mitotic NE breakdown (Guttinger et al., 2009) and during viral capsid egress (Park and Baines, 2006); this raises the intriguing possibility that aPKC functions similarly in muscle cells to remodel the lamina for RNP granule export. Speese et al. follow the RNPs by live-cell imaging and show that they do indeed exit the nucleus. Interestingly, the foci often appeared to be completely surrounded by membrane while inside the nucleus. Imaging of this compartment by electron microscopy revealed that DFz2C-positive granules were present in vesicles in the perinuclear space between the INM and ONM. Granules were also found in invaginations of the INM and evaginations of the ONM, thus tracking a pathway similar to that herpes virus capsids take as they progress through the NE (Johnson and Baines, 2011). Thus, these RNP granules apparently exit the nucleus not through NPCs but rather by remodeling the NE.

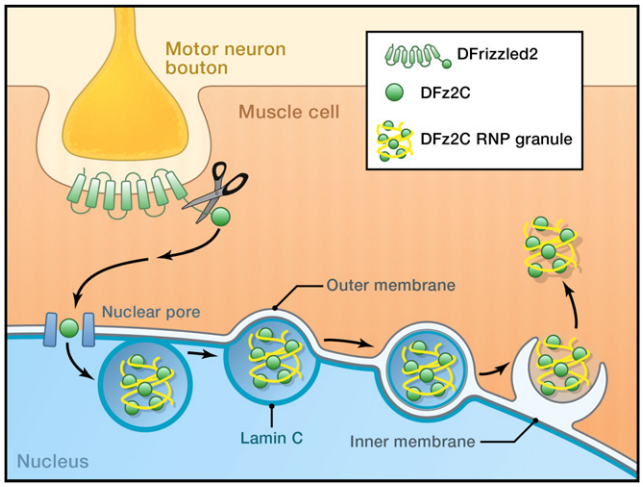

Figure 1. Budding Leads to Nuclear Egress in *Drosophila* Muscle Cells

Formation of the *Drosophila* neuromuscular junction involves synapsis between a motor neuron and a muscle cell, and requires signaling through the wnt receptor DFrizzled2 (DFz2). In the muscle cell, DFz2 is internalized and cleaved near the C terminus to yield DFz2C, which is transported into the nucleus. In the nucleus DFz2C forms granules with lamin C and ribonucleoprotein complexes containing mRNAs; some of which contribute to successful synapse formation. The granules exit the nucleus by passing through the nuclear membranes. Granules are enveloped by the inner nuclear membrane, which pinches off into the perinuclear space, and then fuses with the outer nuclear membrane to access the cytoplasm. The mRNAs may then be trafficked back to the neuromuscular junction for on-site translation.

The NE and lamina function in many nuclear processes such as transcription regulation and chromatin organization (Dechat et al., 2008). Thus, extensive remodeling of the NE for RNP export would likely impact important nuclear functions, and it makes intuitive sense that large RNPs would undergo conformational changes to pass through the NPCs rather than disrupt the NE. Why, then, do DFz2C granules use an alternative transport mechanism? The DFz2C RNP granule likely forms to facilitate transport of mRNAs to the synapse and to restrict translation to the cellular periphery. It is known that when the Balbiani ring particle passes through the NPC, the conformation change allows ribosomes to bind the mRNA as it enters the cytoplasm (Daneholt, 2001). If passage through the NPC of the DfzC2 granule stimulates similar interactions, it would severely compromise its ability to protect the mRNA.

The observed requirement of lamC for granule formation also suggests a new function for lamins in nuclear export. In addition, the requirement of aPKC for foci formation suggests that phosphorylation can regulate remodeling of the lamina as well as its dissociation. It will be interesting to see how lamC stimulates granule accumulation and whether it helps scaffold the granule or promotes transcription of the packaged mRNAs. In addition, if lamins are required for the export of specific RNPs, it could explain the tissue specificity of laminopathies, a pleiotropic group of diseases that are often caused by mutations in the human lamin A/C gene. In support of this, the authors show that expression of a lamin A/C mutant known to cause muscular dystrophy can disrupt RNP granule formation in *Drosophila* muscles (Speese et al., 2012).

Although the characterization of export via NE budding in eukaryotic cells is very intriguing, many open questions remain. It is unclear whether NE budding and NPC-mediated transport are mutually exclusive; the authors do not exclude the possibility that some DFz2C RNPs are translocated through NPCs. It will also be interesting to see how prevalent NE budding is in different systems. The authors note that perinuclear granules similar to the DFz2C foci have been observed in several tissues, but whether they are in the process of being transported is unclear (Speese et al., 2012). In addition, identifying proteins required for the association of DFz2C granules with the nuclear membrane and for its transport through the NE will provide new insights into how the structure of the nuclear membranes is maintained. RNP budding does suggest, however, that similar to other membrane systems in the cell the NE is a dynamic compartment that can undergo substantial remodeling outside of mitosis.

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EGFR in Limbo

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The epidermal growth factor receptor (EGFR) is normally activated by ligand-induced dimerization. Oncogenic mutations in EGFR promote activation in a largely ligand-independent manner. Shan et al. uncover a partially disordered state of EGFR kinase, providing evidence that oncogenic mutations counteract this intrinsic structural instability to promote dimerization and aberrant activation.

Epidermal growth factor receptor (EGFR, also called ERBB1 or HER1) is a receptor tyrosine kinase that, upon binding of extracellular epidermal growth factor (EGF) proteins, dimerizes, stimulating its intrinsic tyrosine kinase activity. Overexpression, amplification, and mutation of EGFR occur in a wide range of human epithelial cancers. Gain-of-function mutations are found in a subset of non-small cell lung cancers (NSCLC), especially those of nonsmoking patients of East Asian heritage, whose tumors often exhibit a bronchoalveolar histology (Sharma et al., 2007) and respond to small molecule EGFR inhibitors. A truncated form of EGFR (EGFRvIII) is associated with tumorigenicity in experimental models and poor clinical prognosis of high-grade glioblastomas. Tumors that harbor EGFRvIII do not respond to approved small molecule EGFR inhibitors and may also provide a mechanism of drug resistance in other cancers (Sok et al., 2006). Indeed, recent work suggests that compensatory EGFR activity contributes to resistance to small molecule inhibitors of oncogenic alleles of BRAF (Corcoran et al., 2012; Prahallad et al., 2012), which can be reversed by coextinction of EGFR and BRAF signaling. Given the clear role of EGFR in the pathogenesis of cancer and the increasingly widespread use of EGFR inhibitors in the clinic, understanding the molecular details of EGFR function will inform the development of improved strategies to inhibit EGFR signaling.

Considerable structural work during the past decade has identified two conformations of the EGFR kinase domain: an active conformation that is similar to other active protein kinases and an inactive state that is reminiscent of an inactive conformation observed in Src family kinases and CDK2 (Stamos et al., 2002; Wood et al., 2004; Figure 1, left). In EGFR signaling, ligand-induced dimerization of the extracellular portion of the receptor drives formation of an asymmetric interaction between the intracellular kinase domains in which the C lobe of the "activator" kinase domain binds to the N lobe of the "receiver" kinase domain (Zhang et al., 2006; Figure 1, right). This regulatory scheme is short circuited by oncogenic mutations, which include various point substitutions, insertions, and deletions (Sharma et al., 2007).

In this issue of *Cell*, Shan and colleagues use long-timescale molecular dynamics to interrogate EGFR kinase activation (Shan et al., 2012). Recent advances in both simulation algorithms and purpose-built computing hardware enable microsecond to millisecond simulations of protein dynamics (Shaw et al., 2010). Such simulations allow molecular voyeurs to "watch" proteins over timescales in which physiologically important conformational changes typically occur. In this study, the authors perform a series of EGFR simulations starting from crystal structures of both active and inactive conformations. This new data suggests